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DEVELOPMENT OF CYATHUS FASCICULARIS, C. STRIATUS, AND CRUCIBULUM VULGARE[1]

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(WITH PLATES I–VI AND THREE FIGURES)

A number of years ago when looking up literature on Gasteromycetes, the writer was impressed by the fact that no researches upon the development of any of the Nidulariaceae had been published since the classic papers of TULASNE (9), SACHS (8), EIDAM (4), BREFELD (1), and DEBARY (2, 3). From time to time, therefore, as suitable stages were found, the materials for these studies have been collected.

Cyathus fascicularis SCHW.[2]

Materials for the study of this form were collected largely in the various commercial greenhouses of Lincoln, Nebraska, where the fungus grows very abundantly during the late winter and early spring upon the wooden flats in which bulbs are planted for forcing. The mycelium is usually well developed upon the flats at the time they are taken from the storage cellars, and the basidiocarps usually mature at about the same time as the flowering of

[1] Contribution from the Department of Botany, University of Nebraska, New Series, no. 32.

[2] Material of this species was sent to Dr. E. A. BURT of the Missouri Botanical Garden for determination. He says: "It is certainly *C. fascicularis* Schw., which differs from the European specimens of *C. olla* Pers. in our herbarium in not having its sporangiales radiately rugose on the side attached to the funiculus. Although this difference is constant in all specimens examined, it is probably too slight for an adequate specific difference." (*C. olla* Pers. = *C. vernicosus* DC.)

the bulbs. By watching these flats, fruit bodies were found in all stages of development.

Materials for morphological studies were fixed in chrom-acetic (Flemming's or Benda's) solutions, dehydrated, cleared in either xylol or bergamot oil, and sectioned in paraffin. The most satisfactory results were obtained with Benda's solution. The basidiocarps were so filled with grit that many fruit bodies were treated with 10 per cent hydrofluoric acid for 48 hours before being dehydrated. The walls of the peridium and peridioles were at best very hard, especially in older basidiocarps, and this, together with the extreme gelatinization of the filaments which takes place during development, made the sectioning very difficult.

CULTURES.—Artificial cultures were first obtained during the early spring of 1914 from material collected at a local greenhouse. The inoculum used was the peridioles from the nearly mature but unopened basidiocarps. The fruit bodies were treated with a 1.5–1000 solution of mercuric chloride for a few minutes; the epiphragm was then removed with sterile tweezers, and the peridioles removed and planted on sterile agar media. Pure cultures were obtained repeatedly in this way, and also by searing the epiphragm with a hot scalpel and then removing the peridioles. An abundant pure white mycelium showing frequent clamp connections developed at once upon any of the ordinary agar media used in laboratories. Fig. 1 shows a culture of this kind which is five days old.

Mycelium in this condition was fixed, stained, and mounted in Venice turpentine. The mycelium branched abundantly, the branches turning at once in the direction of the growth of the main branch, and coming to lie near and parallel to the main branch. The mycelium was very constantly binucleate, and showed abundant clamp connections.

Mycelium from the agar cultures was transferred to sterile loam, old leaves, half rotten wood, etc., in flask cultures. On such media the mycelium made a very vigorous growth, and strong mycelial strands developed (figs. 2, 4–8). Cultures usually dried out before the formation of basidiocarps, and so water was added from time to time. In this way individual cultures were kept growing for several years. When cultures were kept in the light

a fresh crop of fruit bodies usually developed after each watering. No basidiocarps were found upon cultures kept in the dark, no matter how long the cultures were allowed to remain there. The best results were obtained by keeping the cultures in the dark for three or four months (or longer), until the culture medium seemed thoroughly permeated with mycelium, and then adding water just before placing them in the light, where fruit bodies developed in a few weeks. The most abundant development of basidiocarps

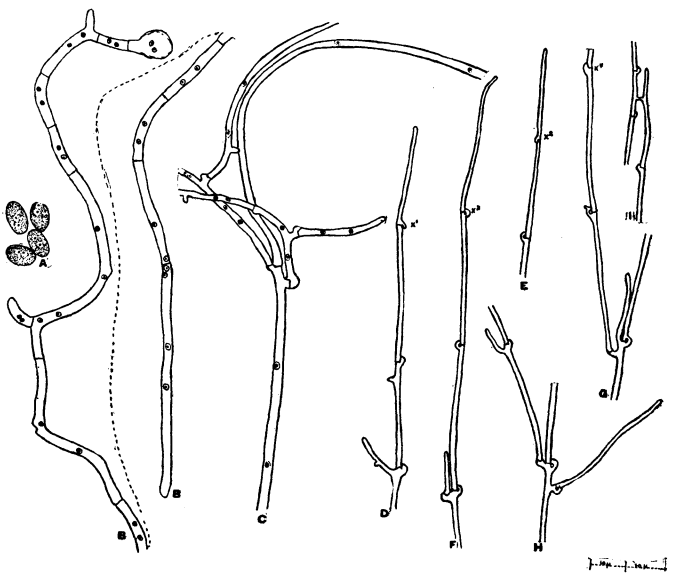

FIG. 1.—*A*, mature spores; *B*, germination spores; *C*, branching and nuclear conditions in older hypha; *D–H*, development of clamp connections (x^1–x^4) and relation of clamps to branching; *I*, anastomosing hyphae.

occurred in cultures exposed in an east window, where direct sunlight reached them during a few hours daily.

HANGING-DROP CULTURES.—Hanging-drop cultures of spores were made repeatedly, but usually spore germination did not take place for some reason. In a few cases, however, especially in one case in which spores from basidiocarps developed in pure cultures were used, abundant germinations in water were obtained. The germ tube usually came from the end of the spore and remained unbranched for some time. In a few cases branching very near

the spore or several germ tubes emerging from the spore were observed. The germinating spores were attached to the cover glass by allowing the water to evaporate until practically dry. They were then fixed in Flemming's fluid, dehydrated, and stained. The spores were constantly binucleate (text fig. 1*A*), and the mycelium developing from them was also composed of binucleate segments or segments which contained paired nuclei (text fig. 1*B*).

Small fragments of the tissue of the peridioles also developed mycelium in the cultures which grew much more extensively. Text fig. 1*C–I* shows the characteristics of the mycelium. In text fig. 1*C*, as well as in most of the mounts obtained where the protoplasmic structure could be determined, the mount was not dried sufficiently to make the mycelium adhere perfectly to the cover glass, so that the direction of growth does not show characteristically. Here, also, there is a binucleate mycelium provided with abundant clamp connections. The clamps appear quite constantly in connection with the branching of the main filaments as well as between the branches. Text fig. 1*D–I* shows in outline portions of a number of such filaments. Text fig. 1*D–G* illustrates the development of the clamps. Near the tip a branch turns backward just above the septum (x^1) and becomes united to the wall just below the septum (x^2). The intervening wall is absorbed (x^3), and a new wall is formed between the clamp and the apical cell from which it arose (x^4). So far as I could determine, there was no evidence that the formation of the clamps was associated constantly with nuclear divisions, as has been described by KNIEP (6) for certain Basidiomycetes. No mounts could be secured, however, in which both walls and nuclei showed distinctly enough in the same specimen to make satisfactory observations on this point. Hyphal anastomoses also occur occasionally (text fig. 1*I*).

MYCELIAL STRANDS.—As soon as growth in culture has reached a few centimeters, the filaments begin to show a tendency to coalesce to form mycelial strands, even upon agar media (fig. 3) and much more conspicuously upon loam, etc. (figs. 4–8). As development continues these strands enlarge until in some cases they become 0.5 mm. in diameter, as found in nature, but usually being 40–200 μ in diameter, the size seemingly being dependent upon the

available nutrition. Figs. 19 and 20 show transverse and longitudinal sections of strands. They are made up of many filaments lying almost parallel to each other and surrounded by a few loosely associated branches which form a vague outer sheath. The cells are very long and 2–5 μ in diameter, the larger being in the center.

ORIGIN OF BASIDIOCARPS.—The basidiocarps originate by the slight differentiation and enlargement of terminal portions of the mycelial strands (figs. 9, 10), and are first discernible in cultures as minute white knots on the mycelium (figs. 5, 6). The filaments of the strand branch in a fanlike manner at the base of the knot (fig. 10), and on the interior above become more slender (about 2 μ in diameter), much branched, and intricately interwoven, making a marked contrast with the filaments of the strand, which are larger and little branched. On the outer tip of the knot, however, is a tuft of hairs the same size as the cells of the mycelial strand. It seems evident, from a study of many slides, that the knot originates slightly below the tip of the mycelial strand, that these terminal hairs are the ends of the filaments which formerly made up the tip of the strand, and that the densely interwoven part just below represents the actual primordium of the basidiocarp. This closely interwoven part is very compact toward the lower part and more open toward the top, where large intermycelial spaces appear.

EARLY INTERNAL DIFFERENTIATION.—The first trace of internal differentiation is the gelatinization of a definite zone of tissue just below the part of the knot which shows the large intermycelial spaces. It extends downward toward the base of the knot in the form of an inverted dome (fig. 11). Fig. 24, between *x*–*x*, shows a higher magnification of this zone. The tissue just to the inside of this zone is made up of small, very densely interwoven filaments, and forms the line of demarcation between the primordium of the gleba and that of the peridium. The glebal primordium is composed of filaments of the same size and general character, but more loosely interwoven, so that large intermycelial spaces remain similar to those found in the earliest stages. Toward the top the filaments become more and more loosely interwoven, and pass

gradually into the large, long, flexuous hairs clothing the tip of the young basidiocarp. Fig. 14 shows a slightly older fruit body in which the gelatinization has progressed much farther, and in which the glebal portion shows much more numerous intermycelial spaces.

ORIGIN AND DEVELOPMENT OF PERIDIOLES.—The peridioles originate in the peripheral portions of the glebal region. They are distinguishable as spots where ends of filaments from all sides converge round a common point, as seen in fig. 12, and more highly magnified in fig. 25 in the region of intersection of lines from *a* to *a*. This mode of origin has also been described by FRIES (**5**) for *Nidularia*. The convergence first takes place in a semicircular manner, extending from the peridium inward toward the central part (fig. 25), but very soon the circle is completed around a common point of convergence. This region is surrounded by a zone of closely interwoven filaments such as originally made up the entire glebal region. A less dense zone with many intermycelial spaces soon tends to form a definite layer within the interlacing filaments, surrounding the converging filaments (except on the under side toward the peridium), and a slightly denser tissue is seen to the outside, which passes into the entirely undifferentiated ground tissue. These faintly marked zones represent the primordia of the walls of the peridiole, while the more or less parallel filaments which interrupt these zones on the under side, toward the peridium, represent the primordium of the funiculus.

The first peridiole to be differentiated is always near the base of the glebal tissue. Other peridioles follow successively from the base of the gleba upward, originating in the same manner from parts of the glebal primordium near its outer part. In the meantime the glebal region has elongated greatly (fig. 13), new growth taking place in an annular region near the apex of the fruit body, where the primordium of the gleba merges with that of the peridium. Fig. 16 shows a higher magnification of a part of the section seen in fig. 13. All the region just described can clearly be differentiated here. The densely interwoven filaments on the outside of the glebal region mark the inner border of the peridium, as will be described later.

As the development of the peridioles progresses, the circle of converging filaments enlarges, due to the multiplication of their elements, largely at the sides, and a central space is observed. This is well shown in figs. 26 and 28, which are higher magnifications of the peridioles in upper and lower portions of the basidiocarp seen in fig. 13. The central space is filled with slime, probably due to the gelatinization of filaments of the fundamental tissue, which are caught in the midst of the more actively growing converging filaments, as in older peridioles remnants of such filaments are clearly present (fig. 29). The cavity in the interior soon takes on an oval or bean shape (figs. 15, 17, 23). This is due to the earlier thickening of the walls of the peridioles on the upper and lower parts, while the sides (ends) remain thinner. Growth remains more active on the sides, and the addition of new elements here, together with mutual pressure, determines the final shape. The enlargement of the cavity in this way continues until the peridiole has reached its mature size.

The converging filaments lining the cavity are at first entirely undifferentiated, appearing in every way similar to the ends of actively growing vegetative filaments such as develop in culture media (figs. 26, 28). Soon they begin to enlarge on the end, and by the time the cavity has reached one-fourth to one-half its ultimate size a definite palisade layer, composed of filaments with enlarged ends, is formed (fig. 29). The palisade layer does not have a uniform even surface as in most of Basidiomycetes, but is made up of basidia and paraphyses of varying lengths. The cells forming the palisade layer are binucleate at first, but the nuclei soon fuse to form the primary nuclei of the basidia and the uninucleate paraphyses (figs. 27, 31). At maturity the entire cavity of the peridiole is densely filled with the oval, constantly binucleate spores (fig. 32).

For some time the walls of the peridioles remain only slightly differentiated (figs. 13, 16). In the subhymenium large intermyceliar spaces remain, and to the outside of this is a denser tissue which will develop into the thick inner wall of the peridiole. Soon, however, the ground tissue of the gleba begins to gelatinize in zones surrounding the already differentiated inner walls of the

peridioles. Figs. 15 and 18 show a basidiocarp where this gelatinization has only taken place around the lower peridioles, while figs. 17 and 21 show a slightly older stage. The tissue remaining between these gelatinizing regions and the denser filaments of the wall of the peridiole becomes the thin, colorless, outer wall of the peridiole. The filaments in this region gelatinize somewhat, but otherwise undergo little change. As the peridioles enlarge, this outer wall becomes stretched out (fig. 23) and remains as a colorless, delicate, easily removable coating over the surface of the mature peridiole. Text fig. 2*a* shows the structure of this layer.

FIG. 2.—Somewhat diagrammatic drawing of wall of mature peridiole through thinner region near side: *a*, outer wall; *b*, pseudoparenchymatic portion of inner wall; *c*, loosely interwoven portion of inner wall; *d*, hymenium.

The cells making up the extreme outer portion of the inner wall of the peridiole become much thickened, pseudoparenchymatic, and brown (fig. 23, text fig. 2*b*), and, showing through the outer hyaline wall, give the characteristic color to the mature peridiole. Within this layer of dark cells is a layer of compactly interwoven filaments whose walls are somewhat gelatinized. This gradually becomes looser as the hymenium is approached (fig. 27, text fig. 2*c*).

In some pure cultures basidiocarps arose between the culture medium and the glass (fig. 4). In such fruit bodies no peridium developed on the side next to the glass, and the whole development of the interior could be followed with a hand lens in an individual basidiocarp. Fig. 6 is an enlargement of the fruit body shown in fig. 4.

DEVELOPMENT OF FUNICULUS.—The funiculus has its origin in the somewhat parallel filaments extending from the innermost surface of the peridium to the primordium of the peridiole. This

appears as a darker area (fig. 25). Later, just to the outside of the inner wall of the peridiole, there appears, between the peridiole and the peridium, a region of actively growing filaments which take the stain readily (fig. 13; higher magnification of left hand peridiole, fig. 28). The filaments in this region elongate rapidly, and soon form a bundle of parallel filaments, as shown in fig. 30, which is a higher magnification of the lower left hand peridiole seen in figs. 15 and 18. Surrounding this bundle are the somewhat gelatinized filaments of the ground tissue, which will form the outer covering of the mature funiculus. The filaments which make up the central bundle of the funiculus continue very active growth, and, being confined by the gelatinizing filaments of the ground tissue, become coiled irregularly in this part. The filaments of the central cord always remain more or less parallel, even in the mature funiculus.

In the mature funiculus (fig. 23) the central cord is attached to the peridiole by the parallel filaments which marked its origin. It immediately twists to form the central coil of the funiculus, and from this passes abruptly at the base into a region of more delicate filaments which merge with the now gelatinizing tissue on the inner wall of the peridium. This central strand is surrounded by the gelatinizing filaments of the ground tissue which constitute its sheath.

The attachment of the funiculus to the peridium is always very weak in this species, so weak, in fact, that as one examines older fruits casually it is not evident, because the peridioles do not seem to be attached. An examination of the peridiole itself, however, shows that the well developed funiculus is always present.

DEVELOPMENT OF PERIDIUM AND EPIPHRAGM.—The mature peridium (fig. 33) is made up of three definite regions, a loosely interwoven outer layer composed of largely longitudinal filaments giving rise to hairs on the surface (*a*), a compact pseudoparenchymatic layer just within this (*b*), and a layer of loosely interwoven, more or less gelatinized filaments which extend from the pseudoparenchymatic layer to the glebal region (*c*).

In the development of the fruit body one can easily trace the origin of these layers. The differentiation takes place first at

the base, as can be observed in sections, and new growth takes place in an apical peripheral zone. This is also clearly shown in text fig. 3*A–D*, where two basidiocarps were marked with India ink, and the position of the lines observed on the older fruit bodies. The outer zone of the peridium comes from the loosely interwoven tissue on the outside of the young knots, which has undergone little differentiation from the condition in the mycelial strand (figs. 11, 12, 17), the zone of pseudoparenchyma, from the closely interwoven filaments within this; while the inner layer is made up of more or less gelatinized filaments which appear very differently at different stages in the development of the basidiocarp. The first differentiation seen in the fruit body is the beginning of gelatinization of the filaments of this inner zone, and in the younger basidiocarps it constitutes the great bulk of the tissue (figs. 13, 15, 17). As the fruit body matures, the continued enlargement of the glebal region causes this inner zone to become more and more compressed, until in a mature specimen it is seen as a zone no wider than the middle zone (fig. 23). At all times the inner zone shows three definite regions so distinctly that they might almost be considered as separate zones. Next to the middle pseudoparenchymatic zone the filaments are loosely interwoven, little if at all gelatinized, and lie longitudinally, following the outline of the middle zone. To the inside these give rise to the wide band of much gelatinized filaments which extend centripetally upward, and merge into the interwoven filaments which border the gleba.

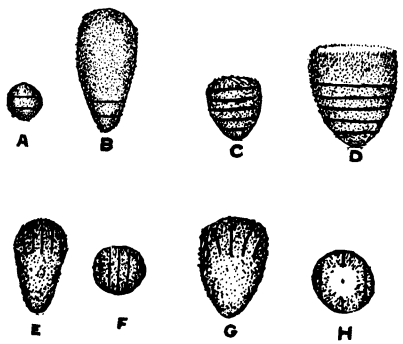

FIG. 3.—*A*, basidiocarp marked with India ink when 3 mm. high to determine region of growth; *B*, same 10 mm. high; *C*, basidiocarp marked when 5 mm. high; *D*, position of marks at time of expansion; *E*, *F*, side and end views of basidiocarp marked just before expansion; *G*, *H*, same basidiocarp with peridium breaking away and epiphragm showing in center.

The peridium covers all of the basidiocarp except the top. To the inside of the abrupt angle shown at the top of the fruit

body (figs. 17, 22) the middle or pseudoparenchymatic layer terminates, and the peridium over the top is composed of the filaments from the outer zone, which here give rise to especially long hairs, and of ungelatinized portions of the inner zone of the peridium.

As the fruit body matures lateral expansion takes place. This causes a rupture in this upper region, the parts structurally connected with the peridium being pulled off from over the top, leaving exposed the little differentiated ground tissue below (figs. 7, 8), which appears as a smooth uniform covering. Text fig. 3*E–H* illustrates how this takes place. A young basidiocarp of about the same age as the one shown in fig. 17 was marked with India ink as illustrated in *E* and *F*, while *G* and *H* show the condition a few days later.

Since the gelatinization of the ground tissue of the gleba begins at the base and progresses upward, the tissue at the top is the last to undergo gelatinization. This, together with the drying effect of the air, results in the formation of a thin membrane, the epiphragm, covering the top, which is exposed by the breaking away of the earlier covering parts. The epiphragm finally ruptures (fig. 5) and itself undergoes gelatinization, leaving the fruit body entirely open on the top. The moisture resulting from gelatinization dries out, and the peridioles sink to the bottom. Thus the basidiocarp assumes its characteristic mature appearance.

Cyathus striatus WILLD.

My studies of this species, based largely upon a small collection of only about 20 basidiocarps made during July 1913 on the campus of the University of Wisconsin at Madison, are necessarily quite incomplete. While this species is quite common in all regions where the writer has collected, it has been impossible to get young stages in most cases. It is included here because it displays some variations in development from that of *C. fasciculatis*, and because of the close agreement with TULASNE'S (9) work upon this species, and because TULASNE shows its close resemblance structurally to *C. fasciculatis*.

The material was fixed in Flemming's medium and weak solutions, dehydrated, cleared in xylol, and sectioned in paraffin.

Cultures of *C. striatus* were made at various times, from material collected near Lincoln, Nebraska, by the same method as used for *C. fasciculare.* An abundant development of mycelium resulted, which was structurally much like that of *C. fasciculare.* The mycelium was at first white, but soon became a dirty brownish color, with many strong mycelial strands. In a few cases small knots appeared upon the mycelium, but mature basidiocarps were never developed.

YOUNG BASIDIOCARPS.—The youngest fruit bodies obtained were 2.5–4 mm. high, and the differentiation had advanced to a considerable degree, so that the peridial and glebal regions were well defined. Figs. 34 and 35 show very low and figs. 37 and 38 higher magnifications of fruit bodies of this type. They show about the same condition as is shown by TULASNE (**9,** *pl. 3, fig. 5*). The peridium has the same structure as in the mature basidiocarp (see fig. 48, the outside to the right), but it is not quite so much hardened. It is made up of three definite zones. The outer of these is made up of loosely interwoven, generally longitudinal filaments, which give rise on the outside to the dense covering of large, stiff, septate hairs about 6–10 μ in diameter; the middle is a pseudoparenchymatic layer which is much wider than the corresponding zone in *C. fasciculare;* while the broad inner layer is composed of more or less gelatinized filaments extending to the central top-shaped primordium of the gleba. This inner zone shows the three definite regions as described for *C. fasciculare.*

At the upper portion of the fruit body is an annular, deeper staining region where the peridial and glebal portions meet (figs. 37–40). This is the region of greatest growth, where new elements are added, both to peridium and gleba, during the elongation of the basidiocarp, just as in *C. fasciculare.* The top of the basidiocarp is covered densely with coarse hairs. The filaments from which these arise are much smaller and lie almost parallel to each other, thus forming a very definite zone over the upper portion of the gleba. The glebal region consists of closely interwoven filaments about 2 μ in diameter, resembling that of *C. fasciculare.*

ORIGIN AND DEVELOPMENT OF PERIDIOLES AND FUNICULUS.—The section shown in figs. 36 and 39 are of a basidiocarp 5 mm.

high when the peridioles are just appearing (cf. TULASNE **9**, *pl. 3, fig. 6*). Their mode of origin is entirely like that of *C. fascicularis* as is shown in fig. 44, a higher magnification of the peridiole seen at the left near the base in fig. 39. The only variation in development is that the peridioles seem to appear simultaneously all through the glebal region. That they show in the photograph at the base is due to chance, for in examining the series it is clearly seen that they are equally distributed in all parts. As differentiation continues, we find the individual peridioles passing through identically the same stages as described for *C. fascicularis*, but while in that species the first peridiole differentiated was at the base and remained somewhat in advance of the others throughout its development, in *C. striatus* the upper peridioles soon outstrip the lower ones, so that the upper ones have formed a definite palisade layer before the lower ones have developed more than a small cavity in the center (figs. 40, 41). The same is true at the time of spore formation, the upper peridioles forming spores abundantly, while the lower ones still show a palisade condition. The gelatinization around the peridioles also takes place from above downward.

The structure of the mature peridiole differs from that of *C. fascicularis* only in its general proportions. All layers are much thinner, but especially is this true of the inner wall of the peridiole, which is often no more than 18 μ thick. Most of the apparent thickness of the wall in fig. 46 is due to the long stalks of the basidia which are shown in fig. 45. At maturity the interior of the peridiole is completely filled with the long, slender, binucleate spores characteristic of this species (fig. 47).

The funiculus also shows much the same course of development as in *C. fascicularis*. In the mature funiculus the filaments making up the coil become much more elongated, and therefore more twisted, and the strands forming the attachment to the peridial wall are more strongly developed. Figs. 41, 43, and 46 show stages in its development.

STRIATIONS ON WALL OF PERIDIUM.—The presence of the striations on the inner portion of the upper part of the peridium of the mature fruit body presents an additional point of interest. As the fruit body reaches maturity, the three layers of the peridial

wall extend practically across the top of the basidiocarp, quite in contrast with the condition in *C. fascicularis*. In this upper portion the peridium becomes folded, as seen in a somewhat tangential longitudinal section (fig. 42). At this time the peridioles reach practically to the top of the fruit body, as in younger stages (fig. 40). At the time of expansion this folded upper portion expands, forming the striate projection above the peridioles, which sink to the base of the fruit body as the gelatinized parts dry out.

Crucibulum vulgare TUL.

As *Crucibulum vulgare* is widely distributed and has been more extensively studied than other species of the Nidulariaceae, the writer was especially interested in obtaining it. The material used for most of my studies was discovered by Miss GERTRUDE E. DOUGLAS on an old gunny sack in the woods on the north shore of Beebe Lake on the Cornell University campus while we were collecting together in July, 1916. The old gunny sack was almost covered with fruit bodies in all stages of development. Knowing that I was especially interested in the group, she kindly turned the collection over to me for fixation and study. Material from this was fixed in Benda's solution, dehydrated, cleared in cedar oil, and imbedded in paraffin. Later more material was collected from an old board fence, also on the Cornell campus, and fixed in chrom-acetic solution. The fixation with this solution was not satisfactory, and little use was made of this collection except for purposes of comparison.

Artificial cultures were made of this fungus also, using the same methods as employed for *Cyathus fascicularis* and *C. striatus*. It made a vigorous growth upon all culture media tried. The mycelium was at first white, but soon became a dirty yellow and in parts quite brownish. Flask cultures, such as proved successful for *C. fascicularis*, were tried, but no mature fruit bodies were obtained. The mycelium showed much the same characteristics as that of *C. fascicularis*. Numerous knots appeared on the mycelial strands and on dense mats of mycelium from time to time, but never matured. MOLLIARD (7), however, obtained mature basidiocarps of *Crucibulum vulgare* in similar pure cultures after two and

a half years. The writer's cultures were never maintained for so long a period.

ORIGIN OF BASIDIOCARP.—The basidiocarps arose from mycelial strands or from densely interwoven vegetative mats of hyphae. Not uncommonly young fruit bodies developed on the inside of old peridia which had lost their peridioles. The youngest basidiocarps obtained were about 0.5 mm. in height and showed no internal differentiation, except that the mass took the stain slightly more deeply at the top where the filaments were much smaller (about 1.5 μ in diameter) and more closely interwoven than at the base. Figs. 49–52 show some of these young basidiocarps, while fig. 65 is a higher magnification of the upper portion of the fruit body shown in fig. 50. At the base of the knot the filaments are larger (about 2.5–3 μ in diameter), where they spring from a mycelial strand or mat, and branch at first in a fan-like manner, soon becoming much branched and interwoven as they ascend, and gradually passing into the smaller filaments at the top. In the region made up of these small, closely interwoven filaments many large intermycelial spaces are present (fig. 65). Even these youngest fruit bodies are covered densely with yellow, much branched, thick-walled hairs with toothlike projections (fig. 65). These hairs are 3.5–4 μ in diameter, and so conspicuous that the smallest basidiocarps found could be distinguished only by the presence of yellow tufts made up of these hairs.

INTERNAL DIFFERENTIATION OF BASIDIOCARPS.—The first differentiation here, as in *Cyathus fascicularis*, consists in the gelatinization of filaments. This zone is very vague in its early stages, and it is difficult to be certain just when it begins. Filaments just beginning to gelatinize seem to take the stain more deeply than ungelatinizing filaments, and soon to lose this power and take the stain less deeply. Using these properties and a very slight difference in appearance as determining factors, it seems that the gelatinization begins at the base of the fruit body (fig. 52) and progresses upward in an annular fashion (fig. 53), the darker regions on the sides representing the region just beginning to gelatinize, and the lighter region below representing the more gelatinized filaments. SACHS (**8**), in his description of the development of

the basidiocarps of *Crucibulum*, also finds that the gelatinization begins at the base and takes place upward in the same manner.

This zone of gelatinization soon becomes a well marked one (fig. 55), extending from the upper peripheral part of the fruit body downward, thus outlining the tissue to the interior, the primordium of the gleba, which has undergone no further differentiation. As the filaments to the outside of the glebal region become more and more gelatinized, they elongate and extend in a downward and outward direction to the outside of the deeply stained filaments bordering the glebal primordium (fig. 55). A pronounced lateral expansion of the basidiocarp results. Fig. 66, a higher magnification of the upper left hand portion of fig. 55, shows the structure of the glebal primordium and the tissues bordering it. This is the condition of the fruit body just before peridiole formation.

Fig. 56 shows a fruit body slightly older than the one shown in fig. 55, with the peridioles just beginning to appear. As in *C. fascicularis* and *C. striatus*, the first trace of peridiole formation is found in the appearance of regions near the outer part of the glebal region, where the filaments point radially toward a common center. Fig. 67 shows a higher magnification of the peridiole primordium seen at the base of the fruit body in fig. 56. These inward pointing filaments soon become surrounded by a layer of densely interwoven filaments, and this layer by a region with many intermycelial spaces, which is only slightly interrupted on the side toward the peridium by filaments which lie more parallel to each other and extend downward (fig. 67) to a deeper staining region just outside the zone with intermycelial spaces. This is the primordium of the funiculus.

The further development of the peridiole takes place much as in *C. fascicularis*, and even in its final differentiation shows only variations in minor details (figs. 56–59, 61–62, 70). The peridioles here, contrary to the observations of SACHS, seem to appear in all parts of the gleba almost simultaneously, as in *C. striatus*, and seem to develop almost uniformly in all parts of the fruit body. The only variation was that the lowest peridiole is quite uniformly smaller and sometimes not so well developed. Fig. 64

shows the interior of a mature peridiole with the cavity almost completely filled with the oval binucleate spores. In thickness and consistency the walls of the peridiole are more like those of *C. fasciculatis*, and like it are much thinner on the ends than on the sides.

In *Crucibulum*, however, the filaments of the outer wall remain entirely ungelatinized for a longer time, and this tissue is more definitely separated from the gelatinizing filaments surrounding it by a thin border layer of filaments which soon become entirely gelatinized. The remnants of these gelatinized filaments bordering the peridioles take the red stains (saffranin and fuchsin) deeply thus giving the very conspicuous border to the outer wall of the peridioles as seen in fig. 70. As can be seen in figs. 58 and 61, much more of the undifferentiated glebal tissue remains and undergoes complete gelatinization than in the species of *Cyathus* studied.

The funiculus has its origin, as already pointed out, in a region of more active growth just to the under side of the young peridiole (figs. 56, 67). In this region the filaments elongate very rapidly, and soon develop into a stout bundle of parallel filaments extending from the young peridiole to the glebal wall (figs. 57, 68). The filaments forming this bundle are surrounded by gelatinizing filaments which extend in the same general direction, and will form the sheath of the funiculus. The central strand of parallel filaments continues its elongation as the basidiocarp develops, and by the time a palisade layer is differentiated (figs. 58, 59) two definite regions are easily distinguishable, a region of much coiled and twisted filaments forming a knot just below the peridiole, and a long slender strand reaching down much below the present position of the peridiole and widening out as it attaches itself to the peridium (fig. 59). This was also described by TULASNE.

These strands are easily traceable far down the sides of the peridium by the conspicuous clamp connections which are abundantly present (fig. 60). The position of the clamps indicates that growth in the strand is upward from the peridial region. Among the gelatinizing filaments surrounding the central strand of the funiculus can clearly be seen the less gelatinized filaments making up the sheath of the funiculus (figs. 61, 62, 70). A funiculus from a nearly mature basidiocarp is shown in figs. 69 and 70.

DEVELOPMENT OF PERIDIUM AND EPIPHRAGM.—The mature peridium (fig. 63) would closely resemble that of *Cyathus fascicularis* and *C. striatus* if the middle pseudoparenchymatic layer were omitted. The outer layer consists of loosely interwoven, largely longitudinal filaments giving rise to long flexuous hairs on the outside. Among these hairs can be seen only rarely, more often toward the top, traces of the much branched, sharp pointed hairs that originally covered the entire fruit body. The filaments comprising the outer part are thick-walled and brownish, becoming thinner-walled and colorless toward the inner portion, and thus merging into the inner layer, which is composed of more or less gelatinized filaments. This peridium covers the sides and converges slightly over the top of unopened fruit bodies (fig. 61).

These zones of the peridium are quite well differentiated before the primordia of the peridioles appear, and the outer zone undergoes little change during subsequent development. On the other hand, the inner zone changes greatly. In the young fruit bodies (figs. 55, 56) it is a wide zone, but as the glebal region develops it becomes more and more compressed (figs. 57, 59), until in the mature basidiocarp it forms an almost indistinguishable layer (figs. 61, 70).

The epiphragm covers the upper surface of the fruit body and is definitely marked by the time the basidiocarp is half developed (fig. 58). It consists of the entirely undifferentiated upper part of the basidiocarp as seen in younger stages (figs. 55, 56), and is as densely covered by the much branched, pointed hairs as was the original young fruit body (figs. 49–54). Following the gelatinization of the filaments surrounding the peridioles, those constituting the epiphragm also undergo gelatinization. The epiphragm just before gelatinization is shown in fig. 62. There is no opening up of the upper portion by the spreading of the superficial layer as described for *C. fascicularis*.

Comparisons

From FRIES'S description of the development of *Nidularia* it evidently has the simplest structure of any of the Nidulariaceae whose development has been studied. Its development seems to correspond very closely with that of *Crucibulum*, except that the

funiculus is entirely lacking. Both are covered at first with toothed hairs, and the internal structures are similar. The zone of gelatinization, which is the first differentiation to be observed, is similar, except that in *Nidularia* it seems to extend over the top of the basidiocarp also. The development in both seems to be basal, as shown by the location of the toothed hairs on the upper portions of mature basidiocarps. The tissues covering the top of the fruit body just before opening are seemingly homologous, but more definitely limited in *Crucibulum*. The structure of the peridium and the origin and development of the peridioles are similar, but in *Nidularia* they appear first at the base, while in *Crucibulum* they appear simultaneously. The structure of the walls of the peridioles, as described by FRIES, is very different seemingly. The close relationship of these forms, however, is very evident.

In *Cyathus* more differences appear, especially in the structure of the peridium and funiculus, but the general type of development is very similar. Of the forms studied, *C. fascicularis* shows closer resemblances to *Crucibulum* than does *C. striatus*. The part of the gleba to become differentiated first and mature first seems to be very variable in the genera and species studied. While in my materials it has seemed to remain quite constant for each species, there is the possibility that it may vary even in the same species.

Summary

1. All three species are easily grown on artificial media. Mature fruit bodies were obtained only in cultures of *Cyathus fascicularis*.

2. The mycelia of all are very similar except for color. Clamp connections are abundantly present, and conspicuous mycelial strands are formed. The cells of *Cyathus fascicularis* are binucleate or composed of segments with paired nuclei.

3. The basidiocarps of *Cyathus fascicularis* and *C. striatus* arise from mycelial strands in all cases observed; while those of *Crucibulum vulgare* may arise from mycelial strands, dense mats of hyphae, or from the interior of old peridia.

4. The primordium of the basidiocarp seems to have its origin slightly below the tip of the strand, and consists of closely interwoven filaments smaller than those of the strand.

5. The first marked internal differentiation in all three consists of the gelatinization of a zone of hyphae in a region that will become a part of the inner wall of the peridium. A zone of closely interwoven filaments just to the inside of this forms the boundary between gleba and peridium.

6. The origin and development of the peridioles is similar in all. Each peridiole originates around a center, toward which the ends of filaments converge. The structure of the walls of the peridioles differs only in relative proportions.

7. The first peridioles to be differentiated in *Cyathus fasciculatus* are toward the base of the gleba, and later other peridioles develop above them. The peridioles at the base mature before those nearer the top of the gleba. In *C. striatus* and *Crucibulum vulgare* the peridioles appear almost simultaneously throughout the glebal region; but the upper peridioles in *C. striatus* mature before the lower ones; while in *C. vulgare* the development is more uniform.

8. The funiculus of *Crucibulum* differs greatly in form from that of *Cyathus*, especially in the length of the strand at the base. The origin is similar in both.

9. The most marked difference between *Crucibulum* and *Cyathus* is in the structure of the walls of the peridia. In *Cyathus* a middle pseudoparenchymatic layer is present which is entirely wanting in *Crucibulum*.

10. During the expansion of the basidiocarp in *Cyathus* the peridium is pulled off from over the glebal region, leaving parts of the ungelatinized ground tissue to form, for a time, a thin covering (the epiphragm). In *Crucibulum* the epiphragm consists of the undifferentiated primordial tissue covered with branched hairs. This undergoes gelatinization at maturity.

11. The spores of all are constantly binucleate.

I wish to express my indebtedness to the late Professor GEORGE F. ATKINSON, who saw a first draft of this paper, for a number of helpful suggestions, to Miss GERTRUDE E. DOUGLAS for the collection of much of the material used in the study of *Crucibulum vulgare*, and to Dr. E. A. BURT for going over the manuscript of

this paper with me and making suggestions, and for the determination of *Cyathus fascicularis*.

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LITERATURE CITED

1. BREFELD, O., Botanische Untersuchungen über Schimmelpilze. Bot. Mitt. über Schimmelpilze **3**:176–180. 1877.
2. DEBARY, A., Beiträge zur Morphologie und Physiologie der Pilze. Leipzig. 1866.
3. ———, Comparative morphology and biology of the fungi, Mycetozoa, and bacteria. pp. 319–322. 1887.
4. EIDAM, E., Keimung der Sporen und die Entdehung der Fruchtkörper bie den Nidularien. Cohn's Beitr. Biol. **2**:221–245. 1876–77.
5. FRIES, R. E., Om utveckligen of fruktkroppen och peridiolema has *Nidularia*. Särtryck ur Swensk. Botanisk Tidskrift **4**: part 2. 1910.
6. KNEIP, HANS, Beiträge zur Kenntnis der Hymenomyceten III. Zeit. Bot. **7**:369–398. 1915.
7. MOLLAIRD, M., Le cycle de développement du *Crucibulum vulgare* Tul. et de quelques champignons supérieurs obtenu en cultures pures. Bull. Soc. Bot. France **56**:91–96. 1909.
8. SACHS, JUL., Morphologie d. *Crucibulum vulgare* Tul. Bot. Zeit. **13**:833–845, 849–861. 1855.
9. TULASNE, L. and C., Recherches sur l'organ d. Nidulariees. Ann. Sci. Nat. Bot. III. **1**:41–107. 1844.

EXPLANATION OF PLATES I–VI

Figs. 1–8 were made with a Century camera and Zeiss-Tessar lens. The photomicrographs were made as follows: figs. 9–23, 34–43, 46, 49–59, 61, 62, with a horizontal Zeiss camera; figs. 19, 20, 24–33, 44, 45, 47, 48, 60, 63, 64, 69, 70, with a Bausch and Lomb vertical camera and Zeiss lenses; and figs. 65–68 with a Bausch and Lomb vertical camera and Leitz lenses.

PLATES I–III

Cyathus fascicularis

FIG. 1.—Culture growing from peridiole, 5 days old; $\times 1\frac{3}{4}$.

FIG. 2.—Young culture growing on wood and loam; $\times \frac{1}{2}$.

FIG. 3.—Tube culture showing beginnings of strand formation; $\times \frac{7}{8}$.

FIG. 4.—Basidiocarps developing between loam and sides of culture jar in which no peridium formed on side next to glass; $\times \frac{1}{2}$.

FIG. 6.—Basidiocarps shown in fig. 4; $\times 2$.

FIGS. 5, 7, 8.—Basidiocarps and mycelial strands formed in cultures, slightly reduced in size.

FIG. 9.—Median longitudinal section of young basidiocarp with mycelial strand from which it developed; ×31.

FIG. 10.—Slightly older basidiocarp, cut somewhat diagonally to show relation of filaments of strand to basidiocarp; ×31.

FIG. 11.—Median longitudinal section of basidiocarp showing origin of inverted gelatinous dome, which is first internal differentiation to take place; ×31.

FIG. 12.—Median longitudinal section of basidiocarp with zone of gelatinization well defined and origin of peridioles evident; ×31.

FIG. 13.—Longitudinal section (median at top, little to one side of center at base) of basidiocarp, with all parts of mature fruit body distinguishable; ×13.

FIG. 14.—Median longitudinal section of basidiocarp intermediate between figs. 11 and 12; ×31.

FIG. 15.—Median longitudinal section of basidiocarp, somewhat older than fig. 13; ×13.

FIG. 16.—Higher magnification of portion of basidiocarp shown in fig. 13; ×31.

FIG. 17.—Median longitudinal section of basidiocarp slightly older than fig. 15; ×13.

FIG. 18.—Higher magnification of portion of section shown in fig. 15; ×31.

FIG. 19.—Transection of mycelial strand; ×225.

FIG. 20.—Longitudinal section of mycelial strand; ×225.

FIG. 21.—Higher magnification of lower peridiole shown in fig. 17; ×31.

FIG. 22.—Higher magnification of upper portion of section shown in fig. 17; ×31.

FIG. 23.—One peridiole, with funiculus and portion of peridium from longitudinal section of basidiocarp; ×31.

FIG. 24.—Higher magnification of gelatinizing zone (x–x) from section shown in fig. 11; ×225.

FIG. 25.—Higher magnification of earliest trace of peridiole differentiation; center of converging filaments at point of intersection of line A–A; ×225.

FIG. 26.—Higher magnification of peridiole from upper portion of fruit body shown in figs. 13 and 16; ×225.

FIG. 27.—Hymenium and subhymenium as seen in peridiole with mature spores; ×225.

FIG. 28.—Higher magnification of peridiole shown at lower left hand side of figs. 13 and 16; ×225.

FIG. 29.—Section of peridiole about one-half mature size, showing structure of hymenium and tissues below it; ×225.

FIG. 30.—Funiculus of peridiole shown in fig. 29; ×225.

FIG. 31.—Much higher magnification of small portion of hymenium from peridiole just beginning to form spores, showing young binucleate basidia and uninucleate basidia; ×585.

FIG. 32.—Mature binucleate spores from same peridiole as fig. 31; ×585.

FIG. 33.—Longitudinal section of peridium of mature basidiocarp: *a*, loose outer layer; *b*, pseudoparenchymatic layer; *c*, inner, loosely interwoven, and more or less gelatinized layer; ×225.

PLATE IV

Cyathus striatus

FIGS. 34, 35.—Median longitudinal sections of youngest basidiocarps found (basidiocarp in fig. 35 bent at base so that section of lateral wall is seen at base); ×14.

FIG. 36.—Median longitudinal section of older basidiocarp with peridioles appearing; ×14.

FIGS. 37–39.—Higher magnifications of basidiocarps shown in figs 34, 35, 36; ×34.

FIG. 40.—Median longitudinal section of still older basidiocarp; ×73.

FIG. 41.—Higher magnification of portion of section shown in fig. 41; ×34.

FIG. 42.—Tangential longitudinal section showing folding of incurved portion of peridium; ×31.

FIG. 43.—Section of nearly mature peridiole; ×34.

FIG. 44.—Higher magnification of portion of section seen in fig. 39 showing converging filaments in early development of peridiole; ×225.

FIG. 45.—Section of peridiole producing spores, showing hymenial layer; ×225.

Fig. 46.—Section of peridiole producing spores and funiculus; ×225.

FIG. 47.—Mature spores; ×360.

FIG. 48.—Section of peridium of mature basidiocarp; ×225.

PLATES V, VI

Crucibulum vulgare

FIGS. 49–52.—Median longitudinal sections of very young basidiocarps; ×31.

FIGS. 53–55.—Median longitudinal sections of basidiocarp showing early stages in gelatinization of filaments bordering primordium of gleba; ×31.

FIG. 56.—Median longitudinal section showing origin of peridioles; ×31.

FIG. 57.—Slightly tangential longitudinal section showing early stage in development of peridiole and funiculus; ×31.

FIG. 58.—Longitudinal section of older basidiocarp; ×13.

FIG. 59.—Section of peridiole before spore formation, and funiculus; ×31.

FIG. 60.—Section of mature funiculus showing clamp connections; ×360.

FIG. 61.—Longitudinal section of nearly mature basidiocarp; ×13.

FIG. 62.—Portion of longitudinal section of older basidiocarp; ×31.

FIG. 63.—Longitudinal section of peridium of mature basidiocarp; ×225.

FIG. 64.—Section of peridiole showing hymenium and interior filled with spores; ×360.

FIG. 65.—Higher magnification of upper portion of basidiocarp shown in fig. 50; ×225.

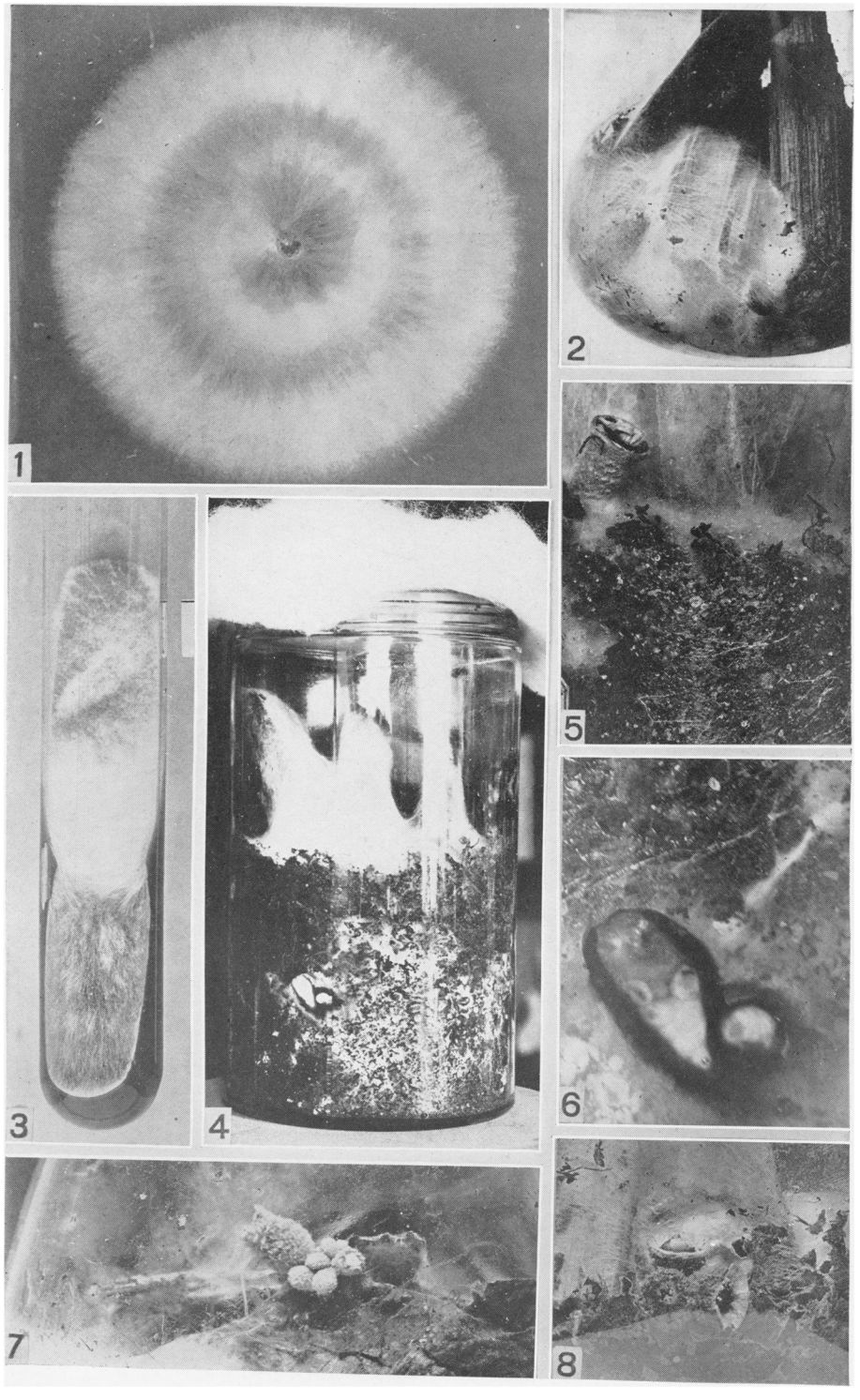
FIG. 66.—Higher magnification of upper left hand portion of fig. 55; ×225.

FIG. 67.—Higher magnification of peridiole initial near base of basidiocarp shown in fig. 56; ×225.

FIG. 68.—Higher magnification of funiculus to peridiole shown to left in fig. 57; ×225.

FIG. 69.—Higher magnification of upper portion of funiculus shown in fig. 70; ×360.

FIG. 70.—Funiculus from another section of basidiocarp shown in fig. 61; ×31.



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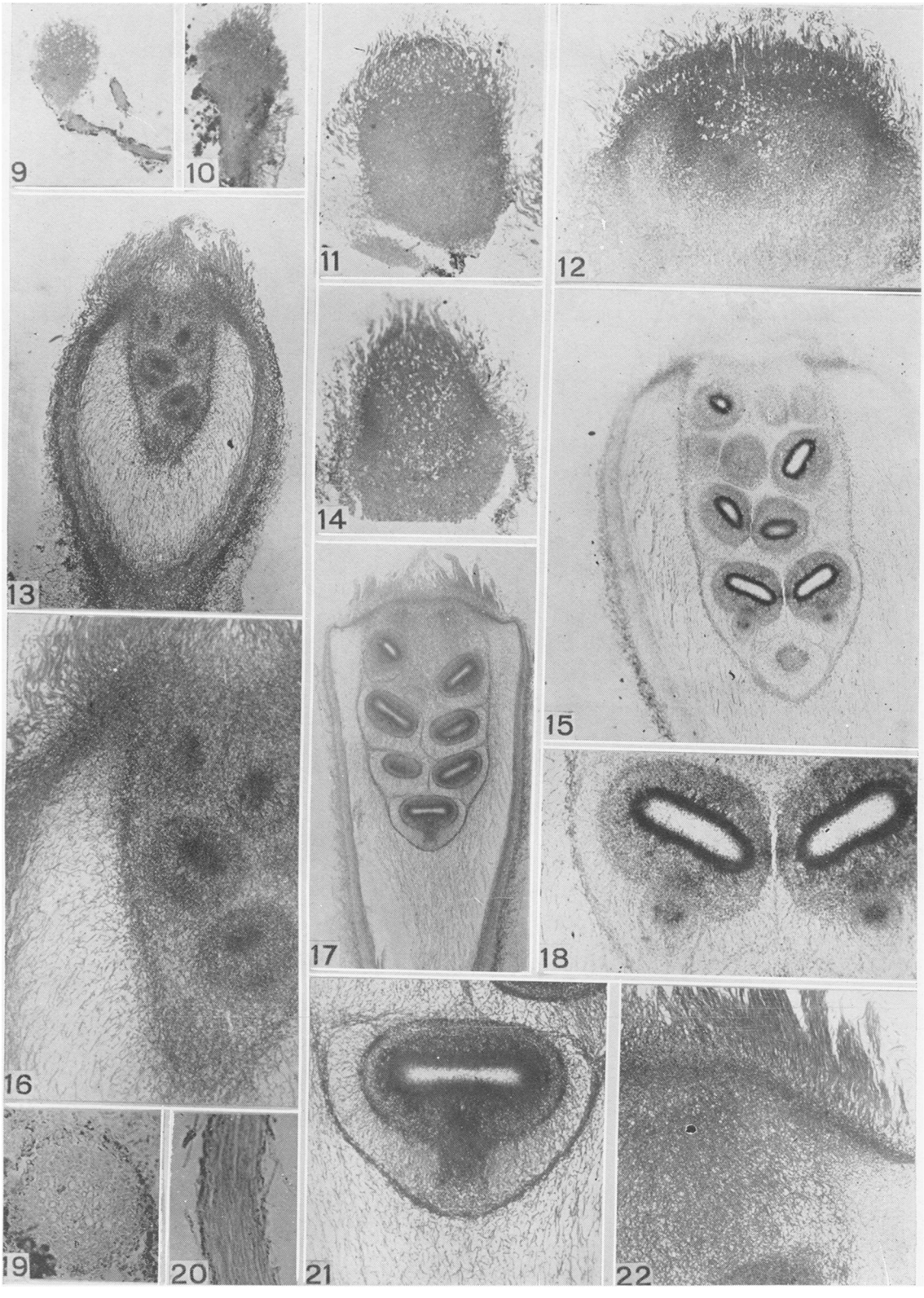

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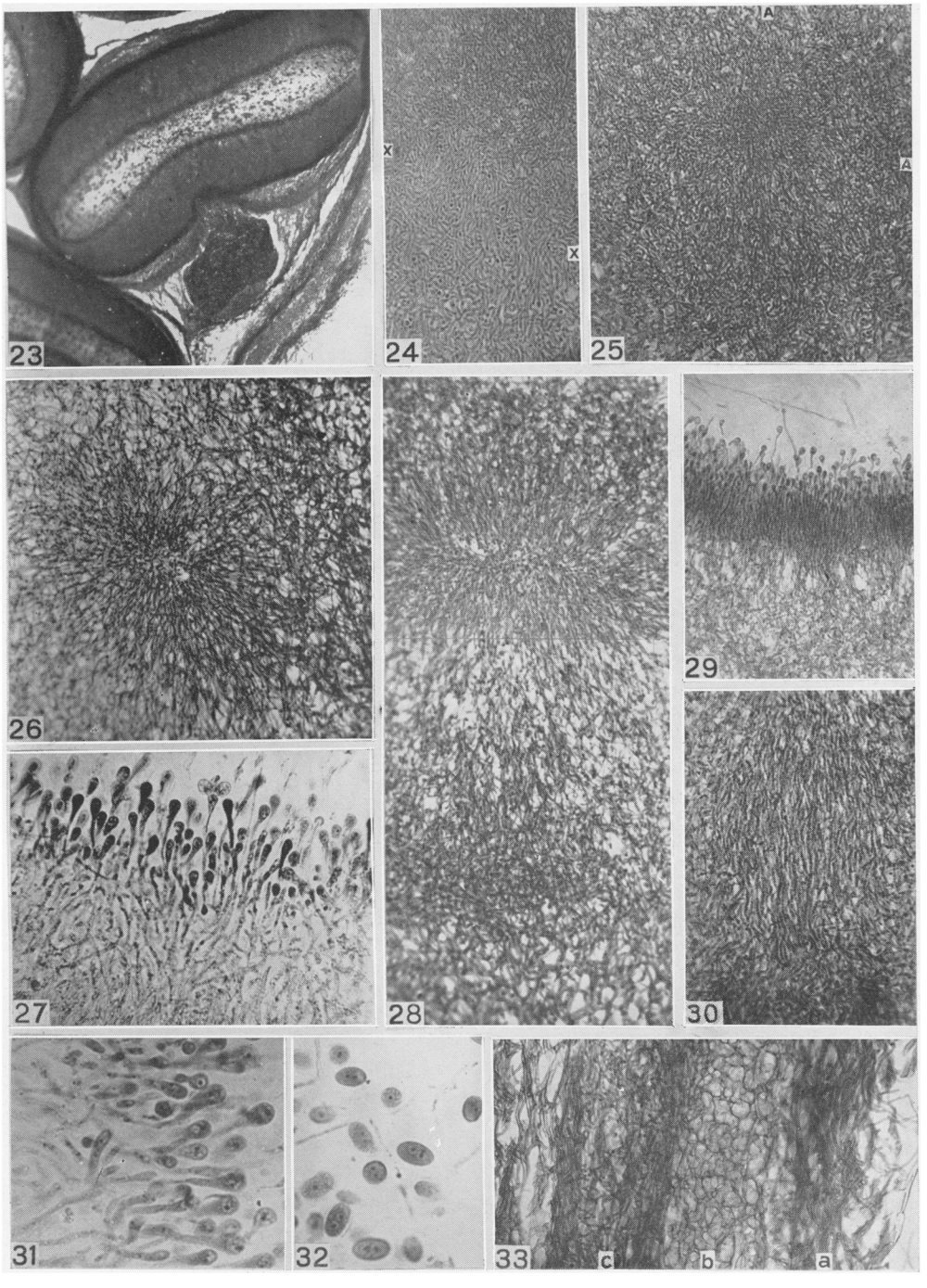

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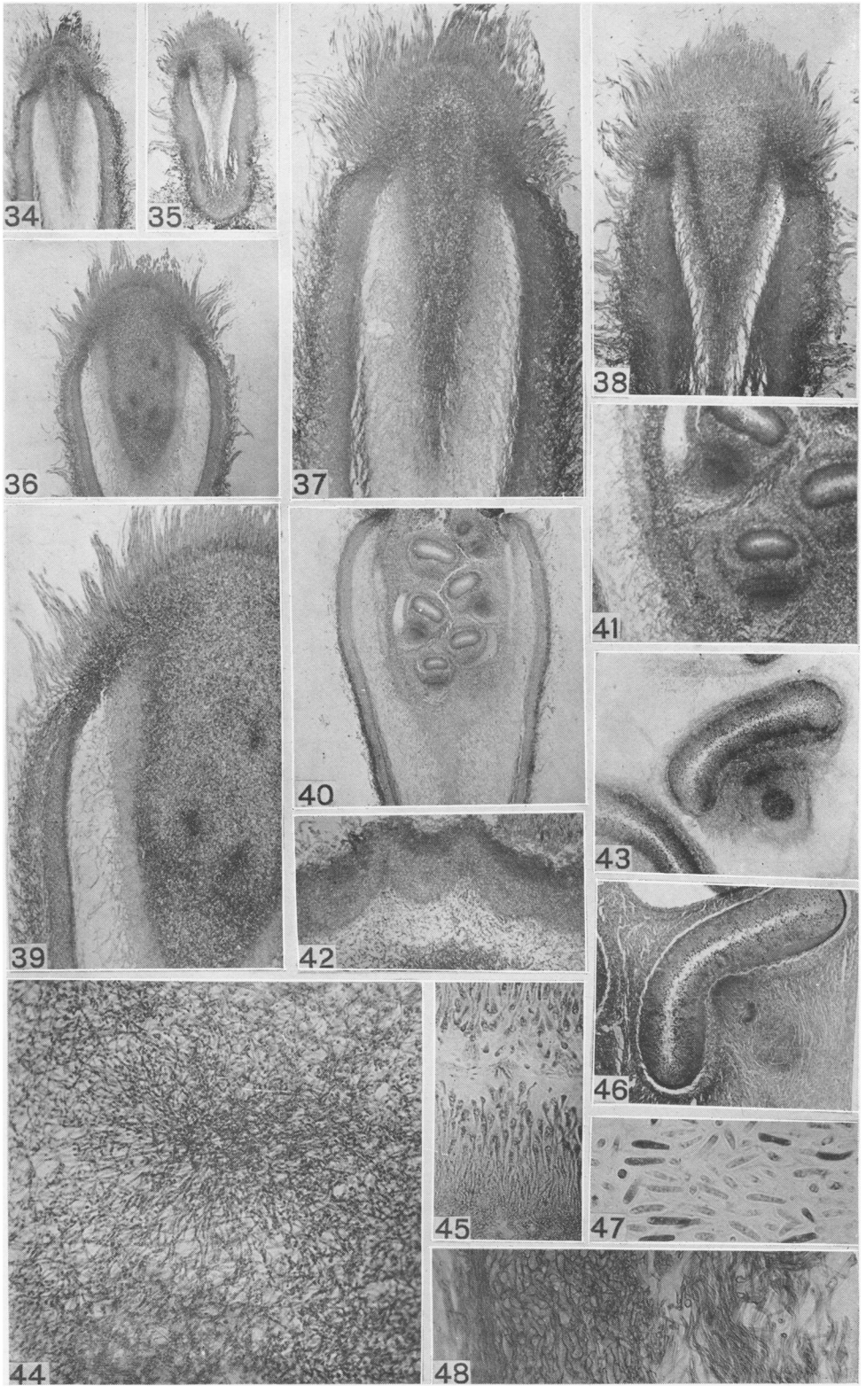

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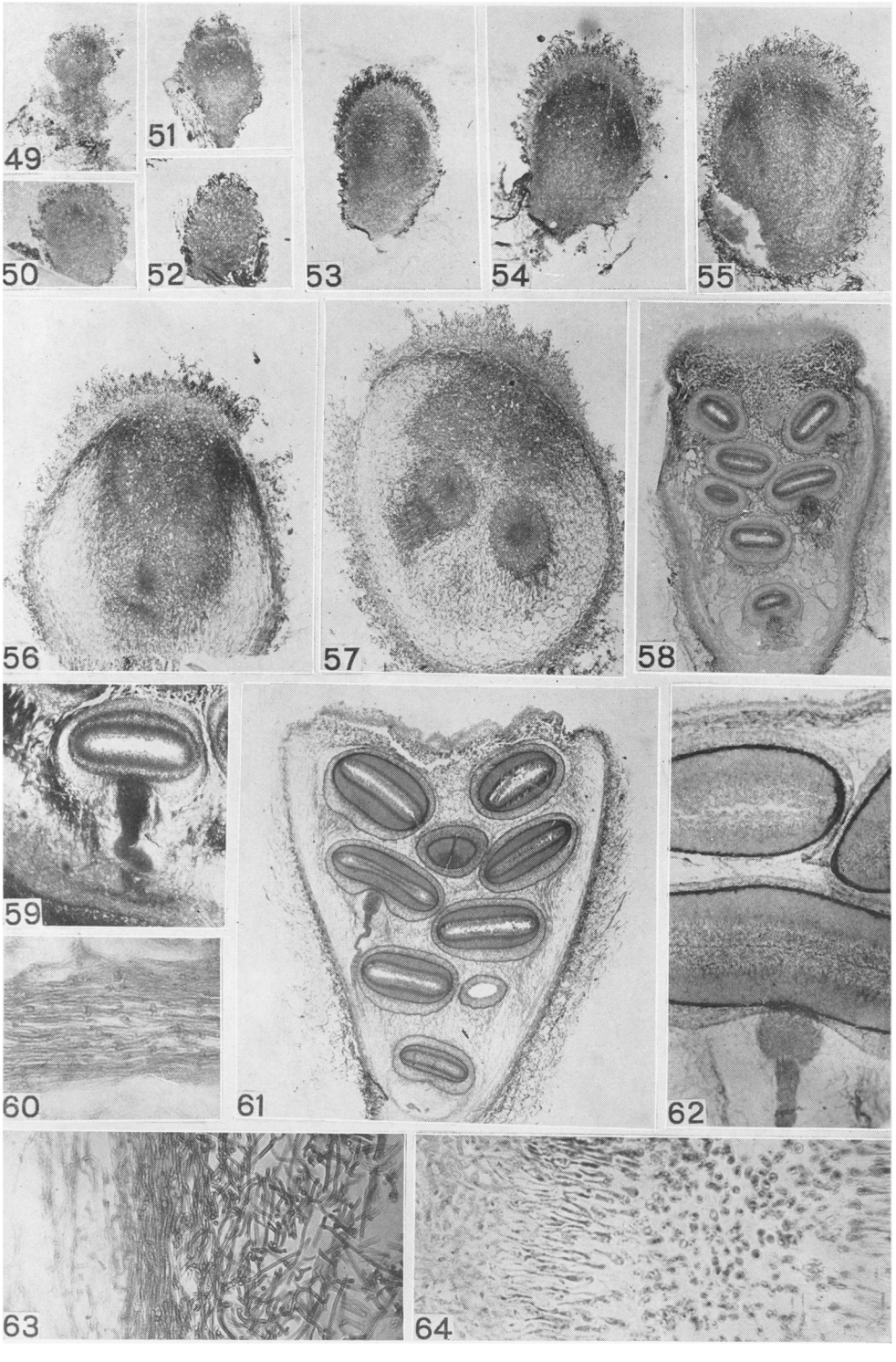

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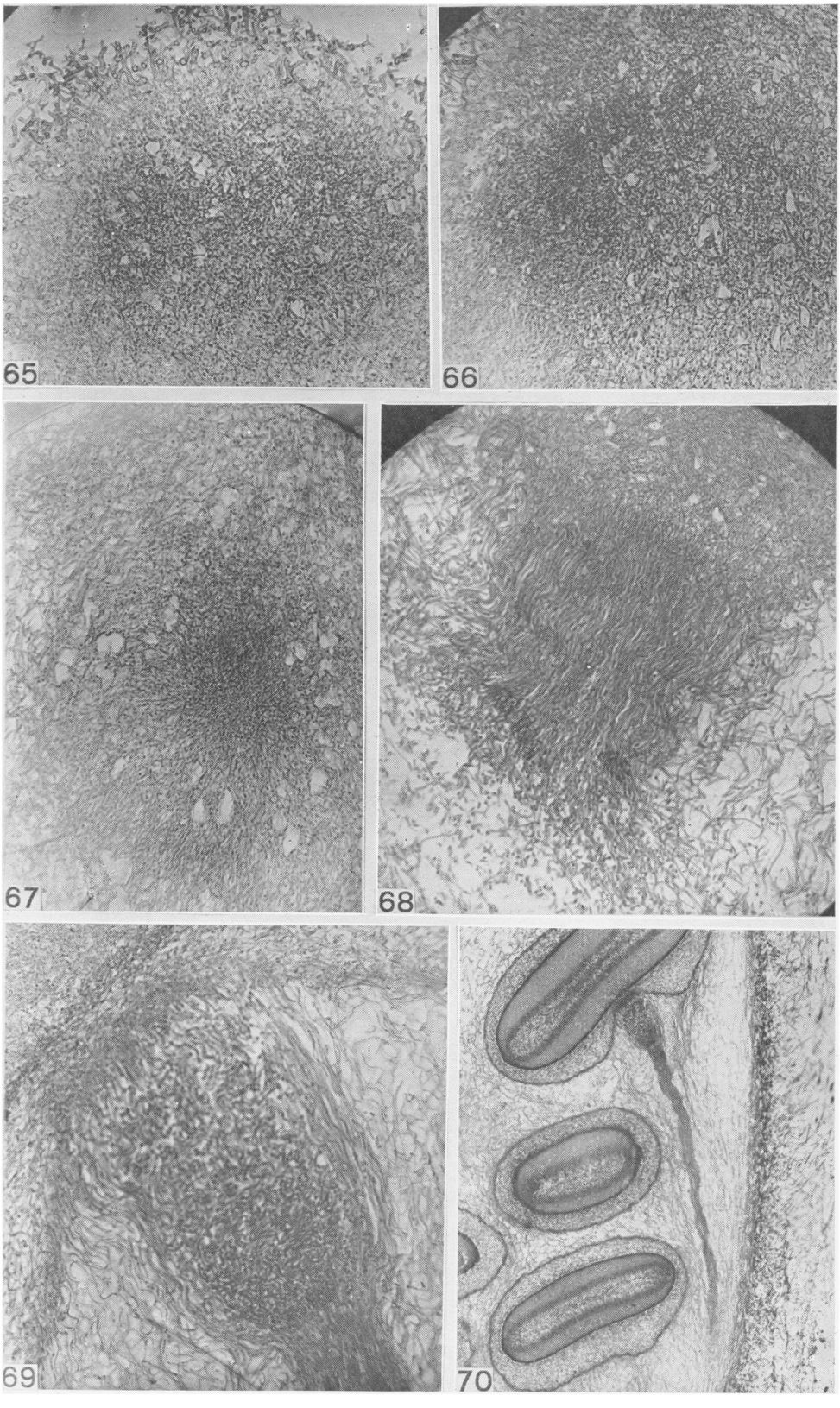
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